

REMARKS

This application has been amended in a manner to place it in condition for allowance at the time of the next Official Action.

Status of the Claims

Claim 1 is amended to include the features previously recited in dependent claims 7, 8 and 11. All of the originally claimed primers are now included in claim 1, since the method is based on the simultaneous determination of p53 and APC gene fragments amplified by those primers. The unifying concept underlying the claimed invention is in fact the possibility of reliably identifying the presence of colorectal tumors or pre-cancerous lesions by carrying out a simultaneous amplification and detection of the eight gene fragments (corresponding to the recited 8 primer pairs, from SEQ ID NO: 1 to SEQ ID NO:16) from both p53 and APC.

Claims 2, 4-8, 11 and 13-22 are cancelled without prejudice.

Claims 1, 9, 10, and 12 remain pending in the application, and include the elected invention and read on the elected species.

**Claim Objections**

The Official Action objects to claims 1 and 13 for reciting "with fluorescent molecule". As this expression is no longer recited, withdrawal of the objection is respectfully requested.

**Claim Rejections-35 USC 112**

Claims 1, 2, 4-6, 8-11, 13-19, 21 and 22 were rejected under 35 USC 112, second paragraph, for being indefinite. This rejection is traversed for the reasons below.

The Official Action rejected these claims for omitting essential steps. However, currently amended independent claim 1 includes the step of determining the reference value as suggested in paragraph 13 of the Official Action, and the term "pre cancerous lesion" has been removed from the claims.

Therefore, pending claims 1, 9, 10 and 12 are definite, and withdrawal of the rejection is respectfully requested.

**Claim Rejections-35 USC 102**

Claims 1, 4-6, 9-11 and 22 were rejected under 35 USC 102(b) being anticipated by SHUBER. This rejection is respectfully traversed.

SHUBER fails to disclose the gene fragments amplified according to method of claim 1, e.g., as previously recited in claim 8.

Therefore, SHUBER does not anticipate claims 1, 9, 10, and 12, and withdrawal of the rejection is respectfully requested.

**Claim Rejections-35 USC 103**

Claim 2 was rejected under 35 USC 103(a) as allegedly being unpatentable over SHUBER in view of ZHOU. Claims 8, 13, 15-19 and 21 were rejected under 35 USC 103(a) as allegedly being unpatentable over SHUBER in view of KMIEC, KMIEC, ALBERTSON, and BUCK. Claim 14 was rejected under 35 USC 103(a) as allegedly being unpatentable over SHUBER in view of KMIEC, KMIEC, ALBERTSON, and BUCK, further in view of ZHOU. These rejections are respectfully traversed.

SHUBER discloses methods of disease detection. These methods are based on PCR amplification, gel electrophoresis analysis and ethidium bromide staining. However, SHUBER fails to recognize a need to improve these methods in terms of test sensitivity and specificity for the detection of colorectal cancer similar to the claimed invention.

For example, ethidium bromide staining and quantification results in a poor discrimination between healthy individuals and colorectal cancer patients. Gel staining does not permit an accurate quantification of PCR amplification and only a few classes can be obtained: null, low, medium or high levels of PCR amplifications (Gastroenterology 119:1219-1227, 2000).

While SHUBER may suggest that "labels, such as fluorescent or radioactive labels, may be used", SHUBER only considers other staining possibilities as simple alternatives without contemplating the possibility of important improvements.

However, as described in the article published in 2004 in Neoplasia, when comparing the "ethidium bromide method" with "fluorescent methods", a sensitivity improvement of about 30% was obtained (Neoplasia, 6:536-540, 2004).

A significant improvement with respect to the methods of SHUBER may be obtained by utilizing the claimed fluorescent markers, i.e., providing an improvement in test sensitivity and specificity for the detection of colorectal cancer. The claimed method determines a continuous scalar evaluation of each case, permitting the correct identification of patients with a PCR amplification level only slightly higher than that of healthy individuals. As a result, one is able to better evaluate PCR amplification levels and to determine more accurately the best cut-off to discriminate between healthy subjects and patients.

Thus, SHUBER not only fails to disclose, but also fails suggest such the unexpected sensitivity improvement.

None of ZHOU, KMIEC, KMIEC, ALBERTSEN, and BUCK is able to remedy these deficiencies of SHUBER for reference purposes.

ZHOU was offered for suggesting the use of florescein to study colorectal cancers. However, ZHOU fails to disclose or

suggest using fluorescein with any of the steps presently claimed.

KIMIEC, ALBERTSON, and BUCK were offered for teaching sequences for which the claimed primers are believed to be "simply represent structural homologs".

BUCK, in particular, was offered for providing evidence of the equivalences of primers. However, Buck refers to sequencing analysis, not to the quantification of DNA levels. The performance and correspondence required for a sequence analysis is different from those needed for a quantification study.

For example, in sequencing analysis, the quantity of PCR amplification is not such a stringent or selective limitation. The main factor to be considered is the specificity of the PCR amplicon and purity in order to obtain a good sequencing result.

For DNA quantity evaluation, however, in addition to having a good PCR amplicon quality, it is also important to have a good linearity of the amplification curve and a good correlation with cycle numbers and cycle temperature.

The newly executed Rule 132 Declaration (included in the Appendix of this amendment) demonstrates that for the purpose of quantification not all primers produce the same results. That is, the declaration shows that different primers with the same genomic regions do not produce comparable results in terms of specificity of colorectal cancer detection. This declaration is

believed to be sufficient and addresses the objections raised in the Official Action.

For example, the two different methods, which utilize different primers, are evaluated in terms of FL-DNA (Fluorescence long DNA) values, i.e., in Table 1, are described in greater detail:

i) the claimed primers listed in the Table at page 2, paragraph [0028] of the publication of the present application US2006/0216713, which are described as "method 1" and

ii) the new series of primers from the same chromosomal regions as those of i), which are listed in the declaration as corresponding to those primers from i), which are described as "method 2".

The data resulting from the two methods demonstrates that the use of a selected series of primers determines the FL-DNA values. The difference in the FL-DNA values for a given series is also indicative of a different ability to discriminate between cancer patients and healthy subjects. In fact, the aim of the FL-DNA method is to identify cancer patients with the highest sensitivity and specificity possible.

Tables 2a/b show the sensitivity and specificity of the two different approaches. In Table 2a, using the primers recited in the claimed invention, it is possible to obtain good sensitivity and specificity with different cut-offs (see, for example, 10-15 or 20 ng cut-offs). Conversely, using the other

primers (Table 2b), sensitivity and specificity suffers. This problem does not allow one to identify an accurate (high sensitivity and specificity) cut-off for colorectal cancer detection.

Thus, not all primers produce the same results. Accordingly, applicants respectfully submit that the above-identified publications, alone or in combination, fail to disclose or suggest sequences recited in independent claim 1, from which claims 9, 10 and 12 depend.

In view of the present amendment and foregoing Remarks, therefore, applicants believe that the present application is in condition for allowance at the time of the next Official Action. Allowance and passage to issue on that basis is respectfully requested.

Should there be any matters that need to be resolved in the present application, the Examiner is respectfully requested to contact the undersigned at the telephone number listed below.

The Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to our credit card which is being paid online

simultaneously herewith for any additional fees required under 37  
C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

Respectfully submitted,

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**APPENDIX:**

The Appendix includes the following item(s):

-a 37 CFR 1.132 Declaration by Daniele Calistri, dated January 19, 2009